Research Article

The Association for Clinical Biochemistry & Laboratory Medicine
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Annals of Clinical Biochemistry
2021, Vol. 58(4) 368–376

DOI: 10.1177/
00045632211006740
journals.sagepub.com/home/acb
SAGE

Assessment of humoral responses in COVID-19 using various quantitative antibody tests

Masatoshi Wakui[1]*, Yoshifumi Uwamino[1,2,*], Toshinobu Kurafuji[3], Masayo Noguchi[3], Akemi Ohno[3], Hiromitsu Yokota[3], Haruhito Kikuchi[1], Naoki Hasegawa[2], Hideyuki Saya[4] and Mitsuru Murata[1]; for the Keio Donner Project Team

Abstract

Background: Quantitative antibody tests are expected to be useful in diagnostics of COVID-19 and investigation of herd immunity against SARS-CoV-2. To make it proper to perform them, understanding of the immunological aspects is critically important. The present study aimed to assess humoral responses in COVID-19 using various quantitative antibody tests.
Methods: Four quantitative antibody tests that are different in targeted antigens, detectable immunoglobulin classes and avidity were used. Diagnosis was confirmed by RT-PCR for SARS-CoV-2 detection. Antibody titres of 117 samples collected from 24 COVID-19 patients and 23 non-COVID-19 patients were measured to evaluate correlations between different tests. For 24 COVID-19 patients, antibody titres measured at various time points after the onset or the RT-PCR diagnosis were subjected to assessment of humoral responses.
Results: Correlations between tests were observed to some degree, although there were discrepancies putatively due to differences in measurement principle. Seronegative COVID-19 was diagnosed for some patients, in whom antibody titres were less than the cut-off value in each test throughout the time courses. IgG seroconversion without prior IgM seroconversion most frequently occurred, while predominance of IgM responses over IgG responses was observed in some severe cases. Viral burdens estimated according to threshold cycle values at the RT-PCR seemed to impact antibody responses.
Conclusions: The results provide insights into the nature of humoral responses to SARS-CoV-2 and diagnostic performance of antibody tests.

Keywords
SARS-CoV-2, COVID-19, quantitative antibody tests, IgG, IgM

Accepted: 11th March 2021

Introduction

COVID-19, an infectious disease due to SARS-CoV-2, is generally diagnosed through the viral genomic RNA detection by RT-PCR testing of nasal or pharyngeal swabs, saliva or sputum.[1] This diagnostic test is highly specific but known to have variation in false-negative rate putatively due to sampling bias and RNase contamination. In addition, reduction of the

[1]Department of Laboratory Medicine, Keio University School of Medicine, Tokyo, Japan
[2]Department of Infectious Diseases, Keio University School of Medicine, Tokyo, Japan
[3]Clinical Laboratory, Keio University Hospital, Tokyo, Japan
[4]Division of Gene Regulation, Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan

*These authors contributed equally to this work and both should be considered first authors.

Corresponding author:
Masatoshi Wakui, Department of Laboratory Medicine, School of Medicine, Keio University, Shinjuku-ku, Tokyo 160-8582, Japan.
Email: wakuism@a6.keio.jp

viral burden by host-defensive mechanisms makes it difficult to detect SARS-CoV-2 using RT-PCR.[2] For symptomatic patient and asymptomatic close contacts in whom the RT-PCR results are negative, serological testing for detection of antibodies against the virus makes COVID-19 diagnosis possible.[1] Quantitative antibody tests allow us not only to diagnose current or past infection but also to measure humoral responses to SARS-CoV-2. To properly perform them for diagnostics of COVID-19 or investigation of herd immunity against SARS-CoV-2, understanding of the immunological aspects is critically important. Several commercial quantitative antibody tests are different in targeted antigens, detectable immunoglobulin classes, or detection methodology. While comparisons and correlations between different tests are of considerable interest for laboratory practice, combining assays different in principle is expected to extend information on antibody profiles. The present study aimed to assess humoral responses in COVID-19 using various quantitative antibody tests.

Materials and methods

Samples

The present study was performed under the approval by the institutional review board (20200059).

The 117 serum samples for the present study were collected from 47 patients undergoing RT-PCR testing for SARS-CoV-2 at our medical institution, including 94 samples derived from 24 patients given COVID-19 diagnosis as described below. The antibody titres of 117 samples were measured to evaluate correlations between different quantitative antibody tests. The residuals of daily clinical samples were used. Serum samples were collected and refrigerated at 4°C for five-days after centrifugalizing for clinical chemistry tests and then the residual volumes of serum were collected and stored at –80°C. Twenty-four asymptomatic or symptomatic patients were RT-PCR positive to receive COVID-19 diagnosis, while 23 patients with fever or acute respiratory symptoms were RT-PCR negative to receive diagnosis of non-COVID-19. One-Step Real-Time RT-PCR assays were performed using either of two methods. One was using BD MAX with BD MAX TNA MMK and BD MAX ExK TNA-3 (Becton Dickenson, Franklin Lakes, NJ, USA). In this method, the two sets individually consisting of forward and reverse primers and a probe, which are named NIID_N1 and NIID_N2, were used. The RT-PCR positivity was defined as threshold cycle (Ct) values being less than 45 cycles. The other was using LightCycler96 (Roche, Basel, Switzerland) and 2019 Novel Coronavirus Detection Kit (Shimadzu, Kyoto, Japan). In this method, the primers and the probes targeted CDC_N1 and CDC_N2. The RT-PCR positivity was defined as Ct values being less than 40 cycles.

Ninety-four of 117 serum samples were serially collected from symptomatic and asymptomatic COVID-19 patients at various time points after the symptomatic onset or after the RT-PCR diagnosis, which were subjected to assessment of humoral responses to SARS-CoV-2 infection.

Quantitative antibody tests

iFLASH Immunoassay Analyzer-based tests using the SARS-CoV-2 IgG and IgM reagents (iFLASH) (Shenzhen YHLO Biotech, Shenzhen, China), which are called the iFLASH-IgG and iFLASH-IgM tests, respectively, in the present study, measured titres of IgG or IgM reactive against both nucleocapsid protein antigen (N-antigen) and spike protein antigen (S-antigen). The cut-off value to distinguish positivity and negativity was defined as the titre of 10 AU/mL in the iFLASH-IgG and iFLASH-IgM tests. An Alinity i system-based test using the SARS-CoV-2 IgG reagent (Abbott Diagnostics), which is called the Alinity-IgG test in the present study, measured titres of IgG reactive to N-antigen. The cut-off value to distinguish positivity and negativity was defined as the titre of 1.4 index defined as the ratio of the sample measurement level to the cut-off level given by the manufacturer (S/C) in the Alinity-IgG test. A Cobas 8000 system-based test using the Elecsys Anti-SARS-CoV-2 RUO reagent (Roche Diagnostics), which is called the Cobas test in the present study, measured titres of antibodies reactive to N-antigen without distinguishing immunoglobulin classes. The cut-off value to distinguish positivity and negativity was defined as the titre of 1.0 cut-off index (COI) designed by the manufacturer in the Cobas test.

Analysis

To evaluate how antibody titres obtained from different tests are related to positive correlation, data from 117 serum samples, of which 94 samples were collected from 24 RT-PCR positive patients and 23 samples were collected from 23 RT-PCR negative patients, were analysed.

With respect to RT-PCR-positive patients, severity grades of COVID-19 (mild; symptomatic patients without hypoxia, moderate; symptomatic patients with hypoxia requiring oxygen therapy, severe; symptomatic patients with hypoxia requiring mechanical ventilation), oxygen requirement (undergoing oxygen supply or not), Ct values of RT-PCR for SARS-CoV-2 N1 and N2 and days from the onset to the sampling

were summarized to assess whether and how these were relevant to antibody titres. The onset date was basically defined as the date on which symptoms (fever, cough, sputum, dyspnoea, olfactory taste disorder and/or sore throat) or chest imaging findings compatible with COVID-19 such as bilateral ground glass opacity in chest computed tomography (CT) appeared. In the case of asymptomatic patients, the onset date was instead defined as the date on which RT-PCR positivity was confirmed.

For statistical analysis, SPSS 25 (IBM, Armonk, NY, USA) and Microsoft Excel software packaged in the Microsoft Office (Microsoft, Redmond, WA, United States) were used, and a P value < 0.05 was considered significant.

Results

Correlations between different quantitative tests to detect antibodies against SARS-CoV-2

Correlations between different tests were observed to some degree, although there were discrepancies putatively due to differences in measurement principle (Figure 1). The highest correlation was observed between the iFLASH-IgG test versus the Alinity-IgG test ($r = 0.886$, $P < 2.85619 \times 10^{-40}$). The lowest correlation was observed between the iFLASH-IgM test versus the Cobas test ($r = 0.346$, $P < 1.30279 \times 10^{-4}$). The distribution of antibody titres measured by the Alinity-IgG test suggested potential saturation of antigens in the kit by excessive antibodies in the sample, although the detail about how to immobilize antigens in the kit has not been disclosed. It seemed to be difficult for the Alinity-IgG test to exactly quantify titres over the index around 8 S/C.

The antibody titres of all 23 patients given diagnosis of non-COVID-19 diseases according to the RT-PCR negativity were less than the cut-off values in each assay, showing no false-positive results. Thus, the specificity of each quantitative antibody test was 100%.

Antibody responses after the symptomatic onset or after the PCR diagnosis for asymptomatic cases

In nine of 24 patients (37.5%), antibody titres were less than the cut-off values in each test throughout the time courses (Table S1). Four of six asymptomatic patients, three of six mild patients, one of four moderate patients and one of eight severe patients exhibited such seronegative results. Seronegative COVID-19 was diagnosed for these patients.

With respect to 15 patients exhibiting seropositive results, the positive rates of the iFLASH-IgG, iFLASH-IgM, Alinity-IgG and Cobas tests were 100%, 73.3%, 100% and 93.3%, respectively (Figures 2 and 3).

IgG seroconversion without prior IgM seroconversion occurred in 13 of 15 seropositive patients (Figure 2). IgM responses were predominant over IgG responses in three of eight severe patients. In four of eight severe patients, maximum antibody titres during the time courses measured by the Cobas test were more than the cut-off value but less than the mean value of those in all 24 patients as well as than the mean value of those in seropositive 15 patients, unlike those measured by other tests (Figures 2 and 4(a)).

Relationship between severity grades or oxygen requirement versus maximum antibody titres

In comparison of ranking among asymptomatic, mild, moderate and severe patients, maximum IgM titres measured by the iFLASH-IgM test throughout the time courses were the highest in severe patients, while maximum IgG titres measured by the iFLASH-IgG and Alinity-IgG tests were the highest in moderate patients (Figure 4(a)). In comparison between asymptomatic or mild patients versus moderate or severe patients, both IgG and IgM titres measured by the iFLASH-IgG, -IgM and Alinity-IgG tests were higher in moderate or severe patients. In comparison between patients with and without oxygen supply, maximum IgM titres measured by the iFLASH-IgM test were higher in patients receiving oxygen supply, while the maximum IgG titres measured by the iFLASH-IgG test also tended to be higher in those without statistical significance (Figure 4(b)). Maximum antibody titres measured by the Cobas test were not associated with severity grades or oxygen requirement unlike those measured by other tests. Although seronegative patients tended to be asymptomatic or mild as well as to undergo medical care without oxygen supply, it was not statistically significant.

Relationship between Ct values at the RT-PCR diagnosis versus maximum antibody titres

The iFLASH-IgG, -IgM and Alinity-IgG tests exhibited statistically significant negative correlation between Ct values for N1 at the PCR diagnosis versus maximum titres of IgG or IgM against SARS-CoV-2 (Figure 5(a)). The iFLASH-IgM also exhibited statistically significant negative correlation between Ct values for N2 versus maximum IgM titres (Figure 5 (b)). Maximum antibody titres measured by the Cobas test were rather reduced in patients with Ct values for N1 and for N2 being 21 or less and 15 or less, respectively.

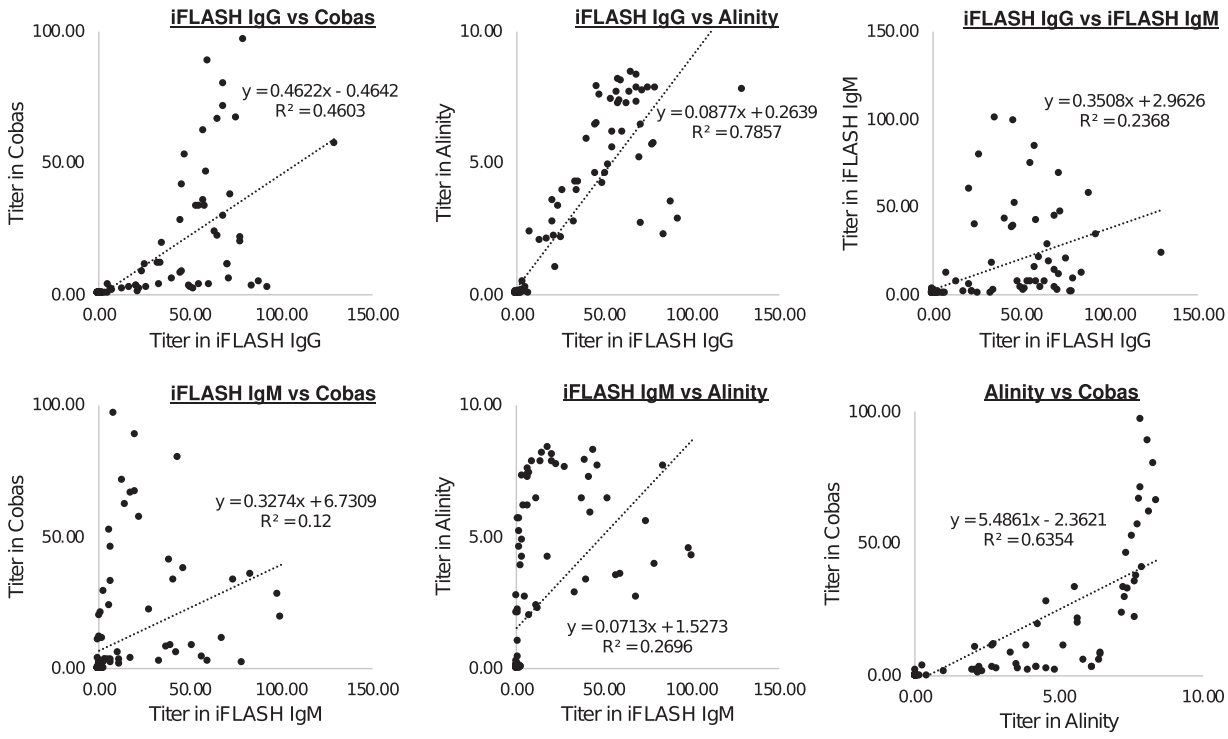

Figure 1. Correlations between different quantitative tests to detect antibodies against SARS-CoV-2. The results of statistical analysis of correlations are as follows: $r = 0.678$ with $P < 4.3017 \times 10^{-17}$ for the iFLASH-IgG test versus the Cobas test, $r = 0.886$ with $P < 2.85619 \times 10^{-40}$ for the iFLASH-IgG test versus the Alinity-IgG test, $r = 0.487$ with $P < 2.65409 \times 10^{-8}$ for the iFLASH-IgG test versus the iFLASH-IgM test, $r = 0.346$ with $P < 1.30279 \times 10^{-4}$ for the iFLASH-IgM test versus the Cobas test, $r = 0.519$ with $P < 1.99752 \times 10^{-9}$ for the iFLASH-IgM test versus the Alinity-IgG test and $r = 0.797$ with $P < 5.90849 \times 10^{-27}$ for the Alinity-IgG test versus the Cobas test.

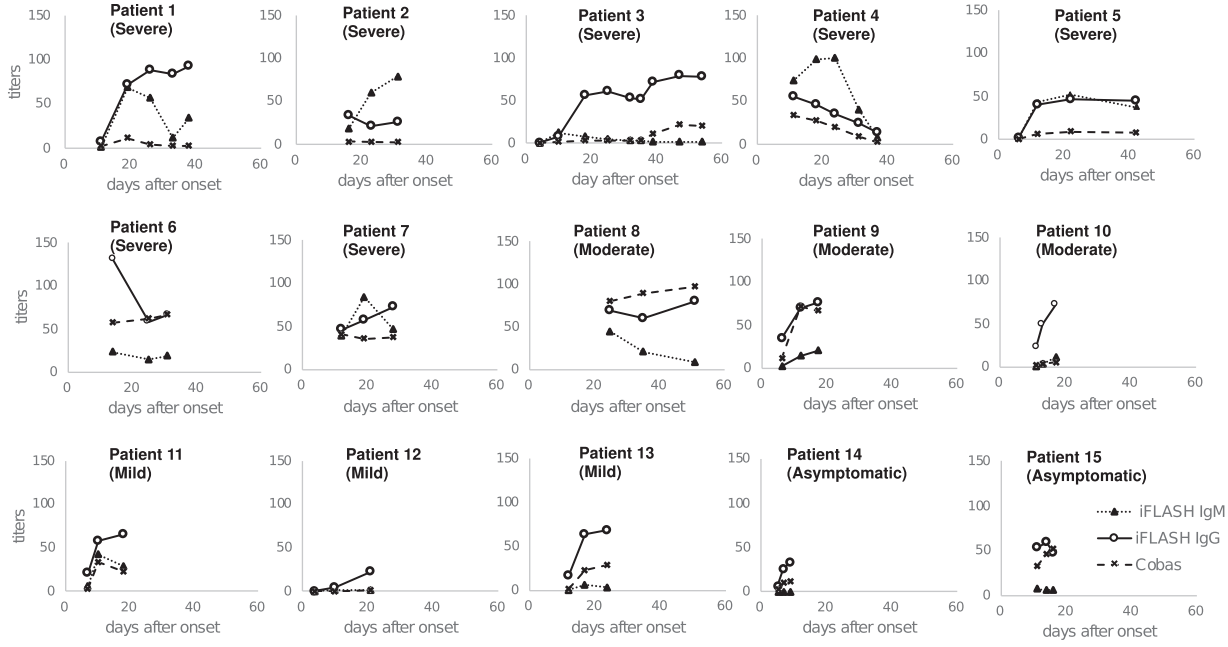

Figure 2. Antibody responses after the symptomatic onset or after the PCR diagnosis for asymptomatic cases revealed by the iFLASH-IgG, iFLASH-IgM and Cobas tests. Antibody titres during the time courses in 15 patients who exhibited maximum antibody titres equalling or exceeding the cut-off value in each test are shown.

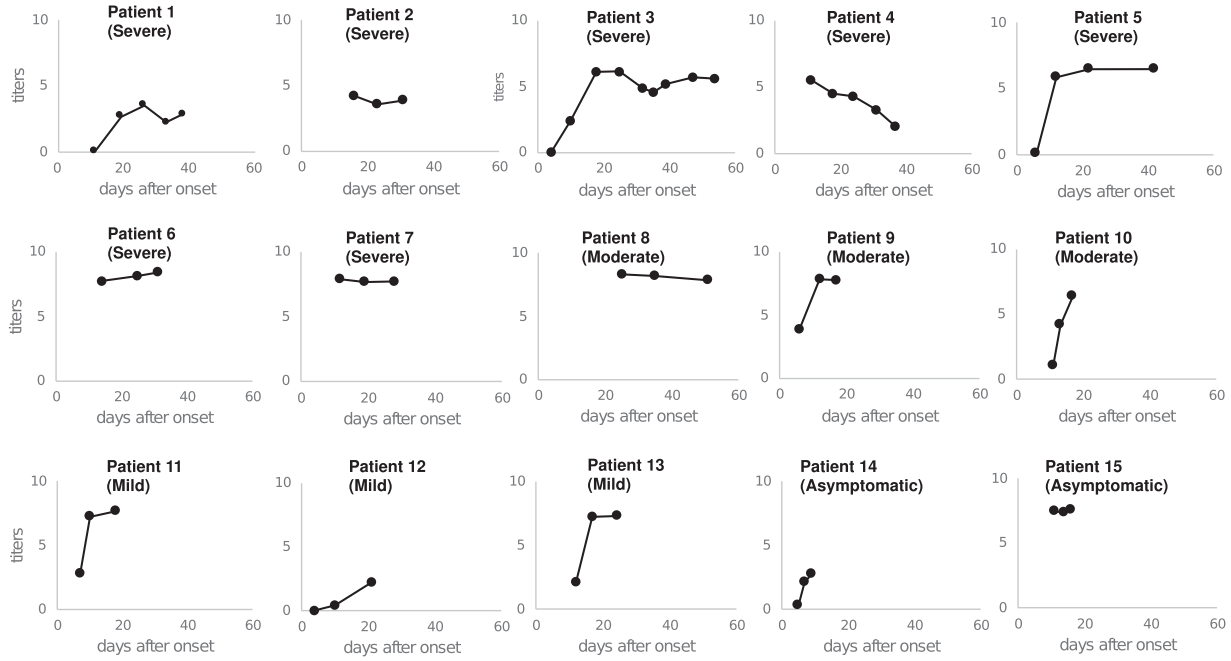

Figure 3. Antibody responses after the symptomatic onset or after the PCR diagnosis for asymptomatic cases revealed by the Alinity-IgG test. Antibody titres during the time courses in 15 patients who exhibited maximum antibody titres equalling or exceeding the cut-off value are shown.

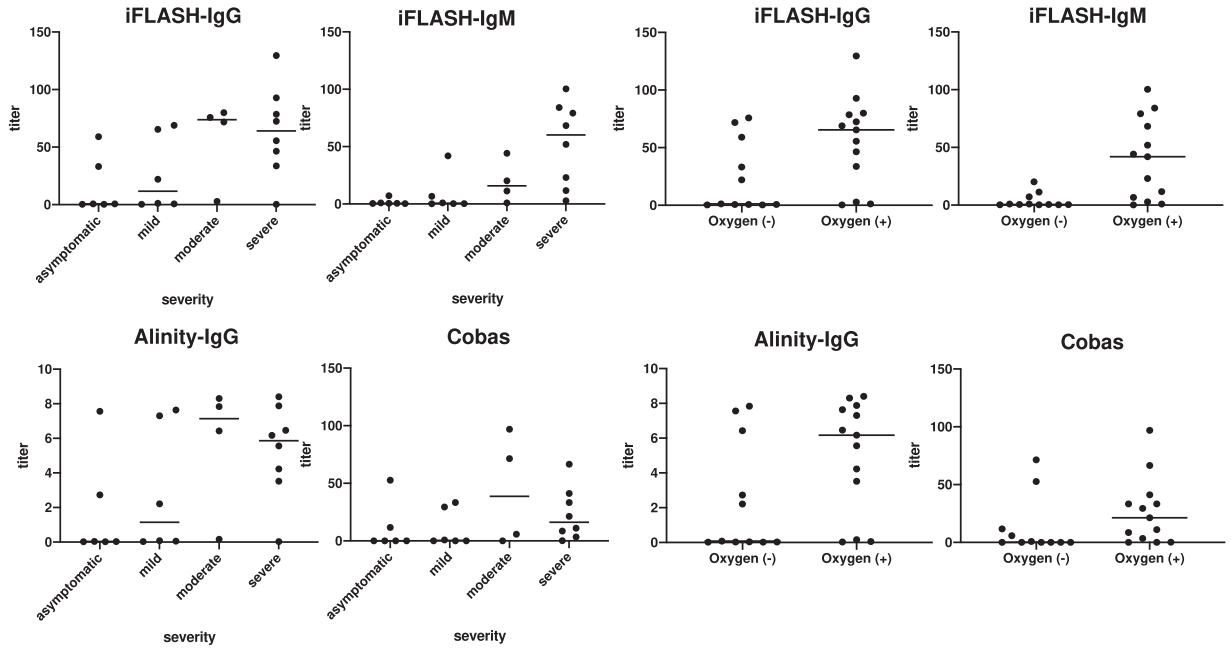

Figure 4. Relationship between severity grades or oxygen requirement versus maximum antibody titres. (a) Maximum antibody titres throughout the time courses in each test are compared among asymptomatic, mild, moderate and severe patients. In comparison of ranking among four groups, maximum IgM titres measured by the iFLASH-IgM test throughout the time courses were the highest in severe patients ($P = 0.0010755$), while maximum IgG titres measured by the iFLASH-IgG and Alinity tests were the highest in moderate patients ($P = 0.0167881$ and 0.0458445, respectively). In comparison between asymptomatic or mild patients versus moderate or severe patients, both IgG and IgM titres measured by the iFLASH-IgG, -IgM and Alinity-IgG tests were higher in moderate or severe patients ($P = 0.00645679$ in the iFLASH-IgG, 0.00411418 in the iFLASH-IgM and 0.02241315 in the Alinity-IgG). (b) Maximum antibody titres throughout the time courses in each test are compared between patients with and without oxygen supply. Maximum IgM titres measured by the iFLASH-IgM test were higher in patients with oxygen supply ($P = 0.00297486$).

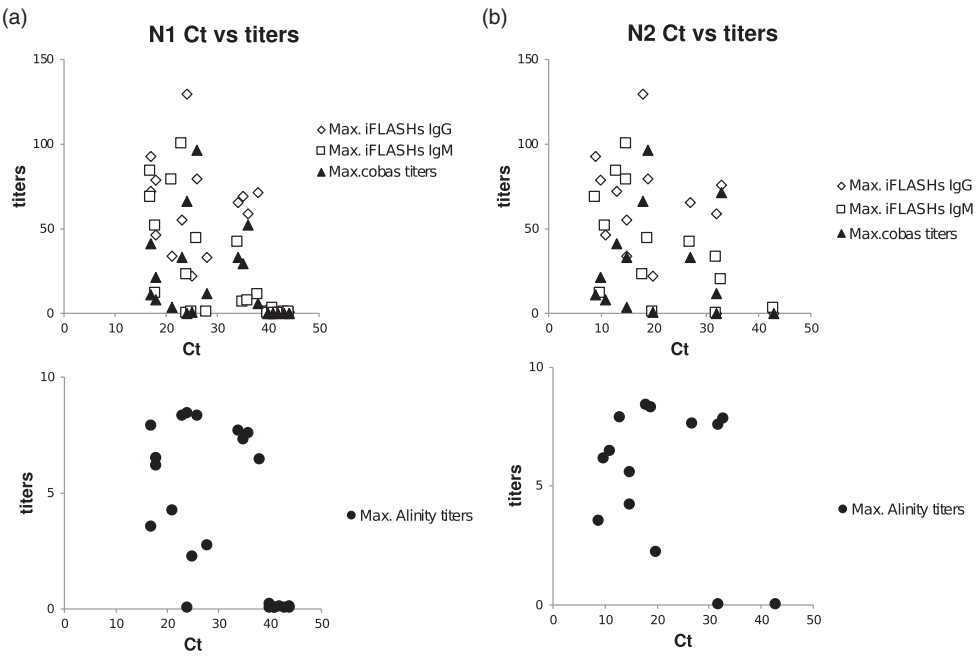

Figure 5. Relationship between Ct values at the RT-PCR diagnosis versus maximum antibody titres. (a) Correlations between Ct values for N1 at the PCR diagnosis versus maximum antibody titres are shown. Negative correlations of Ct values for N1 were significantly observed with maximum titres measured by the iFLASH-IgG, -IgM and Alinity-IgG tests ($r = -0.622$ with $P = 0.01743$, $r = -0.683$ with $P = 0.007123316$, and $r = -0.579$ with $P = 0.030133922$, respectively). (b) Correlations between Ct values for N2 at the PCR diagnosis versus maximum antibody titres are shown. A negative correlation of Ct values for N2 was significantly observed with maximum titres measured by the iFLASH-IgM test ($r = -0.575$ with $P = 0.031474496$).

Discussion

The iFLASH tests measure IgG or IgM against both N- and S-antigens of SARS-CoV-2. This test allows us to compare humoral responses during the time course after the onset or after the initial PCR diagnosis between IgG and IgM without distinguishing the reactivity to N-antigen from that to S-antigen. The Alinity-IgG test measures titres of IgG targeting N-antigen. In the test, it seems to be difficult to accurately measure titres over the index around 8 S/C, while positivity and negativity can simply be determined according to the cut-off value defined as the index of 1.4 S/C. The iFLASH-IgG test results correlated with the Alinity-IgG test results, although the latter seemed to have limitations regarding quantitative evaluation of antibody responses during the time course. The Cobas test is thought to preferably measure titres of anti N-antigen antibodies having mature avidity without distinguishing immunoglobulin classes because of using a double antigen sandwich method.[3] In double antigen sandwich method-based assays, both immobilized antigens and soluble antigens are used to detect specific binding to antigens of interest without detection of immunoglobulin classes. In principle, the detection of antibodies seemingly needs their higher avidities, compared with the detection by conventional immunoassays such as the iFLASH-IgG, iFLASH-IgM and Alinity-IgG tests. In these tests, immobilized antigens and soluble antibodies against human immunoglobulin Fc regions are used to detect specific binding to antigens of interest and to distinguish immunoglobulin classes, respectively. Unlike them, the Cobas test is inapplicable to profiling of humoral responses varying by immunoglobulin class. Combining quantitative antibody tests, which are different in targeted antigens, detectable immunoglobulin classes and detection methodology might aid in understanding of the properties of humoral responses to SARS-CoV-2 infection. However, it remains to be addressed whether antibodies detected by double antigen sandwich method-based assays truly have higher avidities, since direct evaluation of antibody avidities has not been performed practically in most cases. It is of considerable interest that measurement using the Cobas test truly represents the levels of high-avidity antibodies.

The results revealed distinct features of antibody responses to SARS-CoV-2. The iFLASH tests demonstrated that IgG seroconversion without prior IgM seroconversion occurred in majority of patients, as reported previously.[4,5] There are at least two possibilities to explain such observations. One is reinfection or primary infection with SARS-CoV-2 following prior

infection with other coronaviruses to lead to cross-reactive immune. Another possibility is non-canonical immune responses to primary viral infection, which may reportedly occur independently of germinal centre formation in peripheral lymphoid tissues.[6,7] In this case, since a time length resulting from the occurrence of canonical class-switch from IgM to IgG in germinal centres is absent, IgG responses might be allowed to occur prior to or simultaneously with IgM responses.

Predominance of IgM responses over IgG responses appeared in some of severe but not asymptomatic, mild or moderate patients. In comparison of ranking among four groups regarding severity, maximum IgM titres throughout the time courses were the highest in severe cases, while maximum IgG titres were the highest in moderate cases. In comparison between two groups regarding oxygen supply, maximum IgM titres were higher in patients with oxygen supply, while maximum IgG titres also tended to be higher in those without statistical significance. Taken together, the observations seemed to be consistent with previous reports suggesting that IgM titres rather than IgG titres would predict severity and aggravation.[8]

Unlike other tests, the Cobas test exhibited no association of measured titres with severity grades or oxygen requirement. It is of note that maximum antibody titres measured by the Cobas test, which is thought to preferably detect antibodies having mature avidity, were rather reduced in some severe cases. The aggravation of diseases might impair avidity maturation of antibodies via dysregulated immune responses such as cytokine storms. More recently, it has been reported that SARS-CoV-2 antibody responses do not predict COVID-19 disease severity, seeming to contradict suggestions about relationship between them from the present study.[9] Notably, in the recent study, the reagent kit used to measure IgG titres was the same as used in the Alinity-IgG test while IgM titres were measured using a laboratory-developed proteome array. It is plausible that not only the difference in methods to measure antibody titres but also the difficulty for the reagent kit to quantitatively detect IgG responses with titres over 8 S/C might make the discrepancy between the recent and present studies.

While it is of considerable interest whether Ct values at the PCR diagnosis, which represent genome copy amounts to estimate viral burdens, are related to antibody responses, preanalytical matters arising from sampling bias and RNase contamination make it difficult to ascertain the accuracy. Although thus the quantitative evaluation of viral burdens by Ct values has limitations, some statistically significant results were obtained regarding relationship between Ct values versus maximum antibody titres.

In the case of high Ct values such as more than 40, it should be ruled out whether false-positive RT-PCR results mislead us into regarding antibody negativity as evidence for seronegative COVID-19. On the other hand, antibody negativity was observed for all five samples collected 3, 10, 24, 35 and 45 days after the positive RT-PCR result in an asymptomatic patient with the Ct values being 24 for N1 and 32 for N2, which were not thought to be false-positive results, in the present study (Table S1). This plausibly provides evidence for existence of seronegative COVID-19 patients. For further understanding of defensive mechanisms against SARS-CoV-2 as well as proper evaluation of herd immunity, it should be elucidated how immune responses occur differentially between seropositive and seronegative COVID-19 patients. Although the statistical significance was not reached, seronegative COVID-19 tended to be associated with asymptomatic or mild but not moderate or severe cases. According to a recent study in China, 18.9% of 37 asymptomatic COVID-19 patients in the acute phase and 40% of 30 those in the convalescent phase were IgG-negative against SARS-CoV-2, while 16.2% of 37 symptomatic COVID-19 patients in the acute phase and 12.9% of 31 those in the convalescent phase were IgG negative. Our results seem to be consistent with that.[10] Dynamics of T cell responses to SARS-CoV-2 and regulation of cytokine network might reportedly be involved in the differences in immunity between seropositive versus seronegative COVID-19.[10–12] It is of note that the cut-off values used in the present study might fail to detect some antibodies with low titres. If available, prepandemic serum samples would be helpful in addressing such a possibility.

Of note, the Cobas test exhibited reduction of maximum antibody titres in the case of Ct values for N1 and for N2 being 21 or less and 15 or less, respectively. The antibody detection is based on a double antigen sandwich method, which is known to preferably detect antibodies having mature avidity.[7] Excessive soluble antigens reportedly suppress germinal centre formation in the peripheral lymphoid tissues.[13] In addition, antibodies produced independently of germinal centres have been reported to have lower avidity.[7] Collectively, an excessive viral burden might impair avidity maturation under some situations.

Because of using no antibody tests specific to S-antigen in the present study, concerns about whether and how antibody responses to viral N and S proteins are distinct remain to be addressed. Most of commercial tests for detecting antibodies against S-antigen use the S1 region or the receptor-binding domain (RBD), which is contained in the S1 region, but not S2. The S1 region including RBD is poorly conserved among

various coronaviruses, while the S2 region is highly conserved like N protein. As expected, a recent study has provided lines of evidence for cross-reactive antibody responses to S2-antigen and those to N-antigen between SARS-CoV-2 and seasonal coronavirus strains.[14] On the other hand, infection with unidentified coronaviruses having the S1 region highly conserved with SARS-CoV-2 might result in cross-reactive antibody responses to S1. Such potential viruses are expected to be close to a bat coronavirus RaTG13, which is the putatively evolutionary precursor of SARS-CoV-2 because of the highest homology.[15] To investigate the possibility of cross-reactive immune responses, it would be helpful to address whether the IgG seroconversion without prior IgM seroconversion against S1 occurs in COVID-19 patients.

Although it is considered the limitation of the present study that imprecise estimates were included due to heterogeneity in the timing for sampling at which results were obtained, as well as due to a small number of patients and samples, the estimation of multiple serodiagnostics using a minimum number of clinical subjects was effective to provide suggestions about how to understand humoral responses to SARS-CoV-2 infection. To our knowledge, the present study is the first to demonstrate that features of antibody responses measured by a double antigen sandwich method-based assay are distinct from those measured by conventional immunoassays. The double antigen sandwich method-based assay, the Cobas test, expectedly prefers to detect high-avidity antibodies (i.e., mature antibodies) rather than low-avidity antibodies (i.e., immature antibodies), as reported previously.[3] Mature antibodies are secreted by long-lived plasma cells that are developed via the germinal centre-dependent pathway, while immature antibodies are secreted by short-lived plasma cells that are developed via the germinal centre-independent pathway.[7] In the present study, the maximum antibody titres measured by the Cobas test lacked positive correlation with the disease severity or the demand for oxygen supply and negative correlation with the Ct values. It would be consistent with suppression of germinal centre formation in severe COVID-19 patients and in mice undergoing exposure to excessive soluble antigens.[13,16] The novelty of the present study might be to provide insights into development and persistence of mature antibodies, although it remains to be ascertained whether antibodies detected by the Cobas test truly have higher avidities. For evaluation of the herd immunity against SARS-CoV-2, it is of considerable interest whether mature antibodies persist despite decline of antibody titres measured by conventional immunoassays such as the iFLASH-IgG and the Alinity-IgG tests. To address the above concerns, basic, laboratory and epidemiological findings should be accumulated relevantly to diagnostics and therapeutics.

In summary, the present study assessed humoral responses in COVID-19 patients using various quantitative antibody tests. Correlations between different tests were observed to some degree, although there were discrepancies putatively due to differences in measurement principle. Seronegative COVID-19 was diagnosed for some patients, in whom antibody titres were less than the cut-off value in each test throughout the time courses. IgG seroconversion without prior IgM seroconversion most frequently occurred, while predominance of IgM responses over IgG responses was observed in some severe cases. Viral burdens estimated by genome copy amounts according to Ct values at the RT-PCR diagnosis seemed to impact antibody responses. The results provide insights into the nature of humoral responses to SARS-CoV-2 and diagnostic performance of various antibody tests for COVID-19.

Acknowledgements

We thank the medical staffs of Keio University Hospital for their great contribution to clinical work on COVID-19.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Shenzhen YHLO Biotech, which is the manufacturer of the iFLASH Immunoassay Analyzer and the SARS-CoV-2 IgG and IgM reagents for it, provided surgical masks for Keio University Hospital free of charge.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This study was funded by research fund for COVID-19 of Keio University School of Medicine.

Ethical approval

Ethical approval for the present study was obtained from the Ethics Committee of Keio University School of Medicine (20200059).

Guarantor

HS.

Contributorship

MW and YU equally contributed to this work, and both should be considered first authors. MW and YU conceived, designed the study, analysed and interpreted the data and wrote the article. TTK performed the assays. MW, YU, TTK, MN, AO, HY, HK, NH, HS and MM discussed the data and critically reviewed and revised the article. All authors have given final approval for this version of the manuscript to be published.

ORCID iD

Masatoshi Wakui https://orcid.org/0000-0002-3953-0872

Supplemental material

Supplemental material for this article is available online.

References

1. Smithgall MC, Dowlatshahi M, Spitalnik SL, et al. Types of assays for SARS-CoV-2 testing: a review. *Lab Med* 2020; 51: e59–e65.
2. Kucirka LM, Lauer SA, Laeyendecker O, et al. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. *Ann Intern Med* 2020; 173: 262–267.
3. Wu FB, Ouyan HQ, Tang XY, et al. Double-antigen sandwich time-resolved immunofluorometric assay for the detection of anti-hepatitis C virus total antibodies with improved specificity and sensitivity. *J Med Microbiol* 2008; 57: 947–953.
4. Padoan A, Cosma C, Sciacovelli L, et al. Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. *Clin Chem Lab Med* 2020; 58: 1081–1088.
5. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020; 26: 845–848.
6. Swanson CL, Wilson TJ, Strauch P, et al. Type I IFN enhances follicular B cell contribution to the T cell-independent antibody response. *J Exp Med* 2010; 207: 1485–1500.
7. Miyauchi K, Sugimoto-Ishige A, Harada Y, et al. Protective neutralizing influenza antibody response in the absence of T follicular helper cells. *Nat Immunol* 2016; 17: 1447–1458.
8. Hou H, Wang T, Zhang B, et al. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. *Clin Transl Immunology* 2020; 9: e01136.
9. Phipps WS, SoRelle JA, Li QZ, et al. SARS-CoV-2 antibody responses do not predict COVID-19 disease severity. *Am J Clin Pathol* 2020; 154: 459–465.
10. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 2020; 26: 1200–1204.
11. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020; 181: 1489–1501.
12. Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* 2020; 584: 457–462.
13. Han S, Zheng B, Dal Porto J, et al. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl) acetyl. IV. Affinity-dependent, antigen-driven B cell apoptosis in germinal centers as a mechanism for maintaining self-tolerance. *J Exp Med* 1995; 182: 1635–1644.
14. Ng KW, Faulkner N, Cornish GH, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science* 2020; 370: 1339–1343.
15. Lv L, Li G, Chen J, et al. Comparative genomic analyses reveal a specific mutation pattern between human coronavirus SARS-CoV-2 and bat-CoV RaTG13. *Front Microbiol* 2020; 11: 584717.
16. Kaneko N, Kuo HH, Boucau J, et al. Loss of Bcl-6-expressing T follicular helper cells and germinal centers in COVID-19. *Cell* 2020; 183: 143–157.